23 Pages

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
DAVID A. FARAH
SHELDON & MAK PC
100 EAST CORSON STREET, THIRD FLOOR
PASADENA, CA 91103-3842

PCT

COMMUNICATION IN CASES FOR WHICH NO OTHER FORM IS APPLICABLE

Applicant's or agent's file reference 16304-1PCT	Date of mailing (*day/month/year*) 13 MAY 2009 **REPLY DUE** No reply due See paragraph 1 below
International application No. PCT/US06/32264	International filing date (*day/month/year*) 18 August 2006 (18.08.2006)
Applicant BIOVENTUES, INC	

1. ☐ REPLY DUE within _____ months/days from the above date of mailing

   ☒ NO REPLY DUE

2. COMMUNICATION:

The previous PCT/IPEA/409 report mailed on 24 September 2008 is vacated in view of the corrected PCT/IPEA/409.

Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer /Terra Cotta Gibbs/ Telephone No. 571-272-0564

Form PCT/IPEA/424 (January 1994; reprint January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
DAVID A. FARAH
SHELDON & MAK PC
100 EAST CORSON STREET, THIRD FLOOR
PASADENA, CA 91103-3842

PCT

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	2 4 SEP 2008
Applicant's or agent's file reference 16304-1PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US06/32264	18 August 2006 (18.08.2006)	19 August 2005 (19.08.2005)

Applicant

BIOVENTUES, INC

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the *PCT Applicant's Guide*.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed invention is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the IPEA/ US	Authorized officer
Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	/Terra Cotta Gibbs/ Telephone No. 571-272-0564

Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 16304-1PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/US06/32264	International filing date *(day/month/year)* 18 August 2006 (18.08.2006)	Priority date *(day/month/year)* 19 August 2005 (19.08.2005)
International Patent Classification (IPC) or national classification and IPC IPC: **C12Q 1/68**(2006.01);**A01N 43/04**(2006.01);**C07H 21/04**(2006.01);**A61K 31/07**(2006.01) USPC: 536/23.1,24.3,24.5;514/44		
Applicant BIOVENTUES, INC		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 14 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____ , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11 January 2008 (11.01.2008)	12 February 2009 (12.02.2009)
Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer /Terra Cotta Gibbs/ Telephone No. 571-272-0564

Form PCT/IPEA/409 (cover sheet)(April 2007)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US06/32264

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into English, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3(a) and 23.1(b))

☐ publication of the international application (under Rule 12.4(a))

☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:
pages 1-46 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ the claims:
pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 47-59, 59/1 received by this Authority on 13 December 2007 (13.12.2007)
pages* _____ received by this Authority on _____

☒ the drawings:
pages 1/5-5/5 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☒ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☒ the claims, Nos. 85-86
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

5. ☐ This report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 70.2(e)).

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US06/32264

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims 31-84	YES
	Claims 1-30	NO
Inventive Step (IS)	Claims 31-84	YES
	Claims 1-30	NO
Industrial Applicability (IA)	Claims 1-84	YES
	Claims NONE	NO

2. Citations and Explanations (Rule 70.7)
Please See Continuation Sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US06/32264

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☒ table(s) related to the sequence listing

   b. format of material

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No
PCT/US06/32264

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:

Continuation for the new matter
The amendment of the description/claim filed 13 December 2007 is objected to under PCT Article 34(2)(b) because it adds matter into the application that goes beyond the disclosure as originally filed. The added matter which is new is as follows:

Claim 1 has been amended to recite, "having between 5 and 50 residues". Applicant's disclosure has support for the phrase, "having between 6 and 50 residues", but does not appear to have support for the phrase,"having between 5 and 50 residues".

Claims 1-84 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

Claims 1-30 lack novelty under PCT Article 33(2) and lack inventive step under PCT Article 33(3) as being anticipated by or obvious over Tsai et al.

Tsai et al. disclose RNA probes and sets of probes specific for the human fetal hemoglobin comprising capture extenders (see page 2 at Branched DNA assay). It is noted that Tsai et al. is silent regarding whether their disclosed probes and sets of probes are suitable for use with a method for isolating miRNAs. However, the burden of establishing whether the probes and sets of probes disclosed by Tsai et al. would have the additional function of being suitable for use with a method for isolating miRNAs under generally any assay conditions falls to Applicant. Where the claimed and prior art products are identical or substantially identical in structure or composition, or are produced by identical or substantially identical processes, a prima facie case of either anticipation or obviousness has been established. When the PTO shows a sound basis for believing that the products of the applicant and the prior art are the same, the applicant has the burden of showing that they are not. Therefore, the prima facie case can be rebutted by evidence showing that the prior art products do not

Supplemental Box

necessarily possess the characteristics of the claimed product. [T]he PTO can require an Applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [her] claimed product. Therefore, it falls to Applicant to determine and provide evidence that the probes and sets of probes disclosed by Tsai et al. would or would not have the additional function of being suitable for use with a method for isolating miRNAs as instantly claimed.

Claims 1-30 lack novelty under PCT Article 33(2) and lack inventive step under PCT Article 33(3) as being anticipated by or obvious over Barken et al.

Barken et al. disclose RNA probes and sets of probes used in hybridization assays (see page 126 at Table 1). It is noted that Barken et al. is silent regarding whether their disclosed probes and sets of probes are suitable for use with a method for isolating miRNAs. However, the burden of establishing whether the probes and sets of probes disclosed by Barken et al. would have the additional function of being suitable for use with a method for isolating miRNAs under generally any assay conditions falls to Applicant. Where the claimed and prior art products are identical or substantially identical in structure or composition, or are produced by identical or substantially identical processes, a prima facie case of either anticipation or obviousness has been established. When the PTO shows a sound basis for believing that the products of the applicant and the prior art are the same, the applicant has the burden of showing that they are not. Therefore, the prima facie case can be rebutted by evidence showing that the prior art products do not necessarily possess the characteristics of the claimed product. [T]he PTO can require an Applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [her] claimed product. Therefore, it falls to Applicant to determine and provide evidence that the probes and sets of probes disclosed by Barken et al. would or would not have the additional function of being suitable for use with a method for isolating miRNAs as instantly claimed.

Claims 1-30 lack novelty under PCT Article 33(2) and lack inventive step under PCT Article 33(3) as being anticipated by or obvious over Radovich et al.

Radovich et al. disclose RNA probes and sets of probes targeted to BCR-ABL (see page 2645 at Figure 1 and second column). It is noted that Radovich et al. is silent regarding whether their disclosed probes and sets of probes are suitable for use with a method for isolating miRNAs. However, the burden of establishing whether the probes and sets of probes disclosed by Radovich et al. would have the additional function of being suitable for use with a method for isolating miRNAs under generally any assay conditions falls to Applicant. Where the claimed and prior art products are identical or substantially identical in structure or composition, or are produced by identical or substantially identical processes, a prima facie case of either anticipation or obviousness has been established. When the PTO shows a sound basis for believing that the products of the applicant and the prior art are the same, the applicant has the burden of showing that they are not. Therefore, the prima facie case can be rebutted by evidence showing that the prior art products do not necessarily possess the characteristics of the claimed product. [T]he PTO can require an Applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [her] claimed product. Therefore, it falls to Applicant to determine and provide evidence that the probes and sets of probes disclosed by Radovich et al. would or would not have the additional function of being suitable for use with a method for isolating miRNAs as instantly claimed.

It is noted that in Applicant's Remarks filed December 13, 2007, Applicants argue that the PCT Authority has not indicated which portion of any of the sequences it used as the basis for rejecting the claims are "a first adapter segment" "a second adapter segment" and "an miRNA binding segment". This argument has been considered, but is not found persuasive because it should be noted that in their disclosure, Applicant's have not specifically defined "a first adapter segment" "a second adapter segment" or "an miRNA binding segment". Therefore, these segments have been broadly defined to be any portion or segment of the sequences used in the rejection. Furthermore, it should be noted that the claims recite the language "capable of". The term "capable of" denotes a latent property, making it unclear if the property following the recitation "capable of" is intended to actually form a part of the claimed invention.

Claims 1-30 lack inventive step under PCT Article 33(3) as being obvious over either Tsai et al., Barken et al., or Radovich et al. in view of 7,122,364 ('364).

Supplemental Box

Furthermore, US Patent No. 7,122,364 teaches the desire to have resulting fragments of DNA have a recognition sequence for the restriction endonuclease EcoRI at the 5' end of the sequence and a BglII sequence at the 3' end. US Patent No. 7,122,364 also teaches, "the hairpin portion of the molecule can be created by inserting into a cloning vector duplicate copies of a short DNA segment, adjacent to each other but in opposing orientation. The double-stranded fragment encompassing this inverted repeat, and including enough flanking sequence to give short (about 20 nucleotides) unpaired 5' and 3' arms, can then be released from the vector by restriction enzyme digestion"; regarding claims 17-22, Tsai et al., at Figures 1-6 teach that the capture probe is immobilized to a solid support.

---------- NEW CITATIONS ----------

WHAT IS CLAIMED IS:

1. A capture probe suitable for use with a method for isolating microRNAs, the capture probe comprising:

a) a first adapter segment having a first adapter segment sequence having between 5 and 50 residues, the first adapter segment comprising a 3' end and a 5' end;

b) a second adapter segment having a second adapter segment sequence having between 5 and 50 residues, the second adapter segment comprising a 3' end and a 5' end; and

c) an microRNA binding segment having an microRNA binding segment sequence, consisting of 18 or 19 or 20 or 21 or 22 or 23 or 24 residues selected from the group consisting of DNA, RNA, chimeric DNA/RNA, DNA analogs and RNA analogs, and capable of hybridizing to a microRNA listed in a public database;

where the microRNA binding segment is substantially complementary to, and capable of hybridizing to, one or more than one microRNA of interest by Watson-Crick base pairing;

where the 5' end of the first adapter segment is connected to the 3' end of the microRNA binding segment; and

where the 3' end of the second adapter segment is connected to the 5' end of the microRNA binding segment.

2. The capture probe of claim 1, comprising a substance selected from the group consisting of one or more than one type of polynucleotide, one or more than one type of polynucleotide analog, and a combination of one or more than one type of polynucleotide and polynucleotide analog.

3. A set of capture probes, where each of the capture probes of the set of capture probes is a capture probe according to claim 1;

where each of the capture probes comprises identical first adapter segment sequences;

where each of the capture probes of the set of capture probes comprises identical microRNA binding segment sequences; and

where each of the capture probes of the set of capture probes comprises identical second adapter segment sequences.

4. A set of capture probes, where each of the capture probes is a capture probe according to claim 1; and

where the set comprises at least one capture probe comprising an microRNA binding segment that is substantially complementary to, and capable of hybridizing to, each

microRNA listed in a single public database.

5. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe and the second capture probe have identical microRNA binding segment sequences; and

where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

6. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe and the second capture probe have identical second adapter segment sequences; and

where the first capture probe has an microRNA binding segment sequence that is different from the microRNA binding segment sequence of the second capture probe.

7. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical microRNA binding segment sequences;

where the first capture probe and the second capture probe have identical second adapter segment sequences; and

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe.

8. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter

segment sequences;

where the first capture probe has an microRNA binding segment sequence that is different from the microRNA binding segment sequence of the second capture probe; and

where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

9. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical microRNA binding segment sequences;

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe; and

where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

10. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical second adapter segment sequences;

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe; and

where the first capture probe has an microRNA binding segment sequence that is different from the microRNA binding segment sequence of the second capture probe.

11. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe;

where the first capture probe has an microRNA binding segment sequence that is different from the microRNA binding segment sequence of the second capture probe; and

where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

12. The capture probe of claim 1, where the first adapter segment, or the second adapter segment, or both the first adapter segment and the second adapter segment are between 6 and 16 residues.

13. The capture probe of claim 1, where the first adapter segment, or the second adapter segment, or both the first adapter segment and the second adapter segment further comprise a sequence that is a polynucleotide synthesis promoter motif for a polynucleotide polymerase, or that is complementary to a polynucleotide synthesis promoter motif for a polynucleotide polymerase.

14. The capture probe of claim 13, where the polynucleotide synthesis promoter motif is a motif for a polynucleotide synthesis promoter selected from the group consisting of T7, SP6, a T3 DNA dependent RNA polymerase, a type 2 RNA polymerase of *E. coli* and single stranded DNA dependent N4 RNA polymerase.

15. The capture probe of claim 1, where the first adapter segment, or the second adapter segment, or both the first adapter segment and the second adapter segment further comprise a restriction site motif.

16. The capture probe of claim 15, where the restriction site motif is acted upon by a restriction enzyme selected from the group consisting of Not I, Xho I, Xma I and Nhe I.

17. The capture probe of claim 1, where the first adapter segment, or the second adapter segment, or both the first adapter segment and the second adapter segment further comprise a solid phase binding group to immobilize the capture probe to a solid phase.

18. The capture probe of claim 17, where the solid phase binding group is at or near the 3' end of the first adapter segment.

19. The capture probe of claim 17, where the solid phase binding group is at or near the 5' end of the second adapter segment.

20. The capture probe of claim 17, where the solid phase binding group immobilizes the capture probe to the solid phase covalently.

21. The capture probe of claim 17, where the solid phase binding group immobilizes the capture probe to the solid phase non-covalently.

22. The capture probe of claim 17, where the solid phase binding group comprises biotin or an analog of biotin.

23. The capture probe of claim 1, where the microRNA of interest is a eucaryotic microRNA.

24. The capture probe of claim 1, where the microRNA of interest is a primate microRNA.

25. The capture probe of claim 1, where the microRNA of interest is a human microRNA.

26. The capture probe of claim 1, where the microRNA binding segment is exactly the complement to the microRNA of interest in both length and sequence.

27. The capture probe of claim 1, where the microRNA binding segment is more than 90% complementary to a segment of the microRNA of interest of the same length as the microRNA of interest sequence.

28. The capture probe of claim 1, where the microRNA binding segment is more than 80% complementary to a segment of the microRNA of interest of the same length as the microRNA of interest sequence.

29. The capture probe of claim 1, where the first adapter segment has a first adapter segment sequence according to SEQ ID NO:1.

30. The capture probe of claim 1, where the second adapter segment has a second adapter segment sequence according to SEQ ID NO:2.

31. A method for isolating an microRNA of interest from a sample comprising the microRNA of interest; the method comprising:

a) providing a sample comprising the microRNA of interest;

b) providing a capture probe comprising:

i) a first adapter segment having a first adapter segment sequence, the first adapter segment comprising a 3' end and a 5' end;

ii) a second adapter segment having a second adapter segment sequence, the second adapter segment comprising a 3' end and a 5' end; and

iii) an miRNA binding segment having an miRNANA binding segment sequence;

where the miRNA binding segment is substantially complementary to, and capable of hybridizing to, one or more than one miRNA of interest by Watson-Crick base pairing;

where the 5' end of the first adapter segment is connected to the 3' end of the miRNA binding segment; and

where the 3' end of the second adapter segment is connected to the 5'

end of the miRNA binding segment;

c) providing a first linker and a second linker;

d) combining the sample, the capture probe, the first linker and the second linker;

e) allowing the first linker to hybridize with the first adapter segment, the microRNA of interest to hybridize with the microRNA binding segment, and the second linker to hybridize with the second adapter segment;

f) ligating the 3' end of the first linker that is hybridized to the first adapter segment to the 5' end of the microRNA of interest that is hybridized to the microRNA binding segment, and ligating the 3' end of the microRNA of interest that is hybridized to the microRNA binding segment to the 5' end of the second linker that is hybridized to the second adapter segment, thereby producing a complex defined as a strand of first linker, microRNA of interest and second linker that have been ligated together (ligated first linker-microRNA of interest-second linker) and that is hybridized to the capture probe; and

g) dehybridizing the capture probe from the strand of the ligated first linker-microRNA of interest-second linker;

where the microRNA of interest has an microRNA of interest sequence, and comprises a 3' end and a 5' end;

where the microRNA of interest is substantially complementary to, and capable of hybridizing to, the microRNA binding segment of the capture probe by Watson-Crick base pairing;

where the first linker has a first linker sequence, and comprises a 3' end and a 5' end;

where the first linker is substantially complementary to, and capable of hybridizing to, the first adapter segment of the capture probe by Watson-Crick base pairing;

where the second linker has a second linker sequence, and comprises a 3' end and a 5' end; and

where the second linker is substantially complementary to, and capable of hybridizing to, the second adapter segment of the capture probe by Watson-Crick base pairing.

32. The method of claim 31, where the sample further comprises one or more than one substance that is chemically related to the microRNA of interest selected from the group consisting of an RNA other than a microRNA and a DNA.

33. The method of claim 31, where the sample is from a eukaryote.

34. The method of claim 31, where the sample is from a primate.

35. The method of claim 31, where the sample is from a human.

36. The method of claim 31, where the sample comprises a tissue or fluid selected from the group consisting of blood, brain, heart, intestine, liver, lung, pancreas, muscle, a leaf, a flower, a plant root and a plant stem.

37. The method of claim 31, where the microRNA of interest consists of 18 or 19 or 20 or 21 or 22 or 23 or 24 RNA residues.

38. The method of claim 31, where the microRNA of interest is listed in a public database.

39. The method of claim 31, where the sample provided comprises a plurality of microRNAs of interest; and

where each of the plurality of microRNAs of interest has microRNA of interest sequences that are identical to one another.

40. The method of claim 31, where the sample provided comprises a plurality of microRNAs of interest comprising a first microRNA of interest having a first microRNA of interest sequence, and a second microRNA of interest having a second microRNA of interest sequence; and

where the first microRNA of interest sequence is different from the second microRNA of interest sequence.

41. The method of claim 31, where the sample provided comprises a plurality of microRNAs of interest comprising a first microRNA of interest having a first microRNA of interest sequence, a second microRNA of interest having a second microRNA of interest sequence, and a third microRNA of interest having a third microRNA of interest sequence;

where the first microRNA of interest sequence is different from the second microRNA of interest sequence;

where the first microRNA of interest sequence is different from the third microRNA of interest sequence; and

where second microRNA of interest sequence is different from the third microRNA of interest sequence.

42. The method of claim 31, further comprising isolating the total RNA from the sample after providing the sample.

43. The method of claim 31, where the capture probe provided is a set of capture probes;

where each of the capture probes comprises identical first adapter segment sequences;

where each of the capture probes of the set of capture probes comprises identical microRNA binding segment sequences; and

where each of the capture probes of the set of capture probes comprises identical second adapter segment sequences.

44. The method of claim 31, where the capture probe provided is a set of capture probes;

where the set comprises at least one capture probe comprising an microRNA binding segment that is substantially complementary to, and capable of hybridizing to, each microRNA listed in a single public database.

45. The method of claim 31, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe and the second capture probe have identical microRNA binding segment sequences; and

where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

46. The method of claim 31, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe and the second capture probe have identical second adapter segment sequences; and

where the first capture probe has an microRNA binding segment sequence that is different from the microRNA binding segment sequence of the second capture probe.

47. The method of claim 31, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical microRNA

binding segment sequences;

where the first capture probe and the second capture probe have identical second adapter segment sequences; and

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe.

48. The method of claim 31, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe has an microRNA binding segment sequence that is different from the microRNA binding segment sequence of the second capture probe; and

where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

49. The method of claim 31, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical microRNA binding segment sequences;

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe; and

where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

50. The method of claim 31, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical second adapter segment sequences;

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe; and

where the first capture probe has an microRNA binding segment sequence that is different from the microRNA binding segment sequence of the second capture probe.

51. The method of claim 31, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe;

where the first capture probe has an microRNA binding segment sequence that is different from the microRNA binding segment sequence of the second capture probe; and

where the first capture probe has an microRNA binding segment sequence that is different from the microRNA binding segment sequence of the second capture probe.

52. The method of claim 31, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe having a first capture probe sequence, a second capture probe having a second capture probe sequence, and a third capture probe having a third capture probe sequence;

where the first capture probe sequence is different from the second capture probe sequence;

where the first capture probe sequence is different from the third capture probe sequence; and

where second capture probe sequence is different from the third capture probe sequence.

53. The method of claim 31, where the first linker segment and the second linker segment comprise a substance selected from the group consisting of one or more than one type of polynucleotide, one or more than one type of polynucleotide analog, and a combination of one or more than one type of polynucleotide and polynucleotide analog.

54. The method of claim 31, where the first linker, or the second linker, or both the first linker and the second linker are resistant to nuclease degradation.

55. The method of claim 54, where the first linker, or the second linker, or both the first linker and the second linker comprise nuclease resistant nucleotides.

56. The method of claim 54, where the first linker, or the second linker, or both the first linker and the second linker comprise nucleotides with a phosphothioate backbone that render the first linker, or the second linker, or both the first linker and the second linker resistant to nuclease degradation.

57. The method of claim 54,where the first linker, or the second linker, or both the first linker and the second linker comprise nuclease resistant nucleotides and comprise nucleotides with a phosphothioate backbone that render the first linker, or the second linker, or both the first linker and the second linker resistant to nuclease degradation.

58. The method of claim 31, where the first linker and the second linker, each comprises between 6 and 50 residues.

59. The method of claim 31, where the first linker comprises at least 10 residues, and at least 10 residues at the 3' end of the first linker are exactly the complement of the corresponding residues at or near the 5' end of the first adapter segment.

60. The method of claim 31, where the second linker comprises at least 10 residues, and at least 10 residues at the 5' end of the second linker are exactly the complement of the corresponding residues at or near the 3' end of the second adapter segment.

61. The method of claim 31, where the 5' end of the first linker, or the 3' end of the second linker, or both the 5' end of the first linker and the 3' end of the second linker comprise a label.

62. The method of claim 31, where the 5' end of first linker comprises one or more than one residue that extends beyond the 3' end of the first adapter segment after the first linker hybridizes to the first adapter segment.

63. The method of claim 62, where the one or more than one residue of the 5' end of first linker that extends beyond the 3' end of the first adapter segment functions as a primer binding site.

64. The method of claim 31, where the 3' end of second linker comprises one or more than one residue that extends beyond the 5' end of the second adapter segment after the second linker hybridizes to the second adapter segment.

65. The method of claim 64, where the one or more than one residue of the 3' end of second linker that extends beyond the 5' end of the second adapter segment functions as a primer binding site.

66. The method of claim 31, where the sample, the capture probe, the first linker and the second linker are combined simultaneously.

67. The method of claim 31, further comprising adding one or more than one RNAse inhibitor to the combination of the sample, the capture probe, the first linker and the second linker.

68. The method of claim 31, where the first adapter segment comprises a solid phase binding group, or the second adapter segment comprises a solid phase binding group, or both the first adapter segment comprises a solid phase binding group and the second adapter segment comprises a solid phase binding group; and

where the method further comprises binding the capture probe to a solid phase before or after combining the sample, the capture probe, the first linker and the second linker.

69. The method of claim 68, where the solid phase is a plurality of paramagnetic particles.

70. The method of claim 68, where the capture probe is bound to a solid phase through the first adapter segment or through the second adapter segment or through both the first adapter segment and the second adapter segment; and

where the method further comprises purifying the capture probes with hybridized first linker, microRNA of interest and second linker--bound to the solid phase by removing non-hybridized first linkers, second linkers and any other substances that are not bound to the solid phase.

71. The method of claim 68, where the solid phase is contained in a vessel comprising a surface and a cap, and where purifying comprises applying a magnetic field to attract the solid phase to the surface of the vessel or the cap of the vessel.

72. The method of claim 31, where the first linker hybridizes to the first adapter segment at a position where the last residue on the 3' end of the first linker hybridizes to a residue on the first adapter segment that is between 1 residue and 5 residues from the 3' end of the microRNA binding segment.

73. The method of claim 31, where the first linker hybridizes to the first adapter segment at a position where the last residue on the 3' end of the first linker hybridizes to a residue on the first adapter segment that is immediately adjacent to the 3' end of the microRNA binding segment.

74. The method of claim 31, where the second linker hybridizes to the second adapter segment at a position where the last residue on the 5' end of the second linker hybridizes to a residue on the second adapter segment that is between 1 residue and 5 residues from the 5' end of the microRNA binding segment.

75. The method of claim 31, where the second linker hybridizes to the second adapter segment at a position where the last residue on the 5' end of the second linker hybridizes to a

residue on the second adapter segment that is immediately adjacent to the 5' end of the microRNA binding segment.

76. The method of claim 31, where the method further comprises purifying the complex.

77. The method of claim 31, where the complex is bound to a solid phase through the first adapter segment or through the second adapter segment or through both the first adapter segment and the second adapter segment; and

where the method further comprises purifying the complex by removing non-hybridized first linkers, second linkers and any other substances that are not bound to the solid phase.

78. The method of claim 31, where the method further comprises purifying the ligated first linker-microRNA of interest-second linker that has been dehybridized from the capture probe.

79. The method of claim 78, where the first linker, or the second linker, or both the first linker and the second linker comprise nuclease resistant nucleotides, or comprise nucleotides with a phosphothioate backbone that render the first linker, or the second linker, or both the first linker and the second linker resistant to nuclease degradation; and

where purifying the ligated first linker-microRNA of interest-second linker comprises applying DNAase to a solution containing the ligated first linker-microRNA of interest-second linker to destroy any DNA present in the solution.

80. The method of claim 78, where purifying the ligated first linker-microRNA of interest-second linker comprises circularizing the ligated first linker-microRNA of interest-second linker.

81. A method for identifying an microRNA of interest, the method comprising:

a) isolating the microRNAs according to claim 31; and

b) sequencing the microRNA of interest portion of the strand of the ligated first linker-microRNA of interest-second linker.

82. The method of claim 81, where sequencing comprises subjecting the strand of the ligated first linker-microRNA of interest-second linker to reverse transcription to produce a double stranded product comprising a first strand of the ligated first linker-microRNA of interest-second linker and a second strand that is the complement of the first strand.

60 59/1

83. The method of claim 81, where sequencing comprises amplifying the double stranded product to produce amplification products.

84. The method of claim 82, where sequencing comprises cloning the amplification products and culturing the amplification products.